FABRICATION OF ANTIBACTERIAL BIO COMPOSITE FROM BACTERIAL CELLULOSE AND *ARECA CATECHU* EXTRACT

KHALILURRAHMAN BIN AZIZAN

BACHELOR OF CHEMICAL ENGINEERING (BIOTECHNOLOGY)

UNIVERSITI MALAYSIA PAHANG

FABRICATION OF ANTIBACTERIAL BIO COMPOSITE FROM BACTERIAL CELLULOSE AND *ARECA CATECHU* EXTRACT

KHALILURRAHMAN BIN AZIZAN

BACHELOR OF CHEMICAL ENGINEERING (BIOTECHNOLOGY)

UNIVERSITI MALAYSIA PAHANG

FABRICATION OF ANTIBACTERIAL BIO COMPOSITE FROM BACTERIAL CELLULOSE AND *ARECA CATECHU* EXTRACT

KHALILURRAHMAN BIN AZIZAN

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

JANUARY 2012

ABSTRACT

Wound can be defined as any physical injury involving a break in the skin that usually caused by an act or accident rather than by a disease. The poor healing wound may slow down the healing process thus will be exposed to the infection of bacteria. To prevent the infection to do not further develop, it is important that the wounds be treated earlier. Therefore, the objective in this study was to produce antibacterial bio composite from bacterial cellulose (BC), starch, glycerol and areca nut extract. This bio composite, besides having the antibacterial properties that are needed for wound healing process, it also possess biodegradable capability. The 25 types of bio composite were fabricated from difference composition of bacterial cellulose (0 wt. %, 7 wt. %, 14 wt. %, 21 wt. % and 28 wt. %) and areca nut extract (0%, 25%, 50%, 75% and 100%).These films will be characterized by using phenolic content test, testing for antibacterial activity, biodegradation by using fungus, soil degradable test, water absorption test, quantitative of tannin test, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM).The antibacterial bio composite showed the presence of phenol and tannin compound by the phenolic content and quantitative of tannin test. The bio composite film that had 100% areca nut extract displayed better antibacterial properties for antimicrobial test against *E. coli* and *S. aureus*. The composite shows the degradable characteristic by the soil degradable test and test of biodegradable by incubate the film with *Aspergillus niger* strain due to the decrease of bio composite weight. For the water absorption test, the highest percentage of areca nut extract and lowest percentage of bacterial cellulose showed the optimum water uptake. From the FTIR test, the bio composite showed the presence of a carbonyl group, hydroxyl group and carbon-oxygen (C-O) bond. An addition, Scanning Electron Microscopy (SEM) was used to observe surface and cross section of the bio composite films. In order to improve the production of antibacterial film, more study should be done to investigate the effect when the film expose to the environment conditions. The fresh areca nut should be used to make sure the phenolic content in the areca nut do not degrade or decrease. Some improvement can be done by study the most suitable solvent that can be used to improve the extraction process. The combination of bacterial cellulose (BC), starch and areca nut extract can be used to produce antibacterial bio composite films which give benefit for wound healing process.

ABSTRAK

Luka ialah setiap kecederaan fizikal yang melibatkan kecederaan di kulit yang biasanya disebabkan oleh tindakan atau kemalangan dan bukan disebabkan oleh penyakit. Kaedah penyembuhan luka yang tidak betul boleh melambatkan proses penyembuhan dan terdedah kepada jangkitan bakteria. Untuk mencegah jangkitan daripada merebak, adalah penting bahawa luka akan diubati awal. Oleh sebab itu, kajian ini bertujuan untuk menghasilkan biokomposit antibakteria dari selulosa bakteria, kanji, gelatin dan ekstrak buah pinang. Bukan sahaja biokomposit ini mengandungi sifat-sifat antibakteria, malah ia juga mempunyai kebolehan untuk terurai. 25 jenis biokomposit dibuat daripada berbeza komposisi Selulosa Bakteria (0g, 7g, 14g, 21g dan 28g) dan ekstrak buah pinang (0%, 25%, 50%, 75% dan 100%).Filem-filem ini akan dikategorikan dengan menggunakan kandungan fenolik, ujian untuk aktiviti antibakteria, ujianbiodegradasi dengan mengunakan tanah dan kehadiran kulat *Aspergillus niger* sebagai agen penguraian, ujian penyerapan air, kuantitatif tanin,*Fourier Transform Infrared Spectroscopy* (FTIR)dan.*Scanning Electron Microscopy* (SEM). Biokomposit antibakteria menunjukkan kehadiran sebatian fenol dan tanin daripada ujian kandungan fenolik dan kuantitatif tanin. Filem biokomposit yang mempunyai 100% ekstrak buah pinang memaparkan ciri-ciri antibakteria yang terbaik untuk ujian antimikrob terhadap *E. Coli*dan *S. Aureus*. Dalam ujian biodegradasi menggunakan tanah dan penguraian dengan kehadiran kulat *Aspergillus niger*, biokomposit menunjukkan sifat kebolehan untuk mengurai berdasarkan kehilangan berat biokomposit Untuk ujian penyerapan air, peratus anekstrak buah pinang yang tertinggi dan peratusan selulosa bakteria yang terendah menunjukkan pengambilan air yang optimum.Dari ujian FTIR, biokompositfilemyang dihasilkan menunjukkan kehadiran kumpulan karbonil, kumpulan hidroksil dan ikatan karbon-oksigen (C-O). Sebagai tambahan, *Scanning Electron Microscopy* (SEM) digunakan untuk memerhati permukaan dan keratan rentas biokompost. Untuk meningkatkan kadar penghasilan filem anti-bakteria, lebih banyak kajian perlu dilaksanakan untuk mengkaji kesan keatas biokomposit apabila didedahkan kepada alam sekitar. Buah pinang yang segar perlu digunakan untuk memastikan kandungan fenolik dan tanin tidak berkurang. Kajian terhadap jenis-jenis pelarut yang terbaik patut dijalankan untuk meningkatkan kualiti proses pengestrakan. Gabungan selulosa bakteria, kanji dan ekstrak buah pinang boleh digunakan untuk menghasilkan filem anti-bakteria biokomposit yang memberi manfaat untuk proses penyembuhan luka.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

^{o}C	Celcius
cm	Centimeter
cm^{-1}	Per centimeter
cm^2	Centimeter square
cm^2/min	Centimeter square per min
g	Gram
g/L	Gram per liter
Gpa	Giga Pascal
h	Hour, Thickness of the film
IR	Infrared
k	Slope
$Kg\ m^{-3}$	Kilogram per meter cube
kV	Kilovolt
M	Molar
M_m	Moisture content at equilibrium
M_t	Moisture content at time *t*
mg/L	Milligram per liter
mg/ml	Milligram per milliliter
min	Minutes
$mL\ min^{-1}$	Milliliter per minutes
mm	Millimeter

nm	Nanometer
W_h	Weight of humid specimens
W_o	Initial weight of specimens
wt	Weight
w/w	Weight per weight
%	Percent

LIST OF ABBREVIATIONS

BC	Bacterial cellulose
CO_2	Carbon Dioxide
D	Diffusion coefficient
DI	Deionizer
FTIR	Fourier Transform Infrared Spectroscopy
OD	Optical Density
RH	Relative Humidity
SEM	Scanning Electron Microscope
PC	Plant Cellulose
TC	Terminal Complex
TPS	Thermoplastic Starch

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Wound can be defined as any physical injury involving a break in the skin that usually caused by an act or accident rather than by a disease. Wounds can be classified as open or closed. An open wound is a break in the skin or in a mucous membrane while closed wound involves underlying tissues without a break in the skin or a mucous membrane. The local effects of an open or closed wound may include loss of blood, interference with blood supply, destruction of tissues, nerve injury, functional disturbances, and contamination with foreign material. From the research conducted by MacKay and Miller (2003), the definition of wound healing is the body's natural process of regenerating dermal and epidermal tissue.

The healing process includes absorption of blood and serum that have seeped into the area, repair of injured cells, replacement of dead cells with scar tissue, and recovery of the body from functional disturbances. The poor healing wound may slow down the healing process thus will be exposed to the infection of bacteria. To prevent the infection for further develops, it is important that the wounds be treated earlier. The process of healing should starts immediately after an injury and may continue for months or years until the wound recovers. The cleanliness of the wound site must be maintained throughout the healing process.

There are a few herbs that have been used as wound healing. Areca nut is one of the popular traditional herbal medicines and widely cultivated throughout Thailand and South Asia. The areca nut is also used for chewing for people in some Asian countries. Karphrom et al. (2009) state that areca nuts contained hydrolysable tannin, condense tannin, some alkaloids and fats. From the research conducted by Buhler and Miranda (2000), the phenolic compounds in areca nuts were reported to have effect on anti-virus and anti-microorganisms.

According to Czaja et al. (2006), bacterial cellulose (BC) has recently attracted a great deal of attention for biomedical application such as artificial skin burn or wound healing material. To broaden the biomedical applications of bacterial cellulose (BC), various attempts have been made to produce bacterial cellulose (BC) bio composites with high functionality (Yasuda et al., 2005). Bacterial cellulose (BC)/areca nut extract bio composite is one of the candidates that have great potential applications as antibacterial bio composite. Bacterial cellulose (BC)/areca nut extract antibacterial bio composite was prepared by adding the areca nut extract in mixture of blend bacterial cellulose (BC), starch, glycerol and water followed by casting process. The antibacterial bio composite aim to restore the milieu required for skin regeneration and to protect the wound from environmental threats and penetration of bacteria. However, bio composite films dressing are better suited for small wound, since they lack absorbance and are impermeable to water vapor and gases (Jonathan and Zilberman, 2009).

1.2 PROBLEM STATEMENT

Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Meenakshi et al., 2006). In other words, wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, hematoma, laceration or an abrasion (Enoch and Leaper, 2005). Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wound and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase (Sumitra et al., 2005). The improper techniques of wound healing will make the wound become worst. To solve this problem, the development of alternative materials for dressing the wound infection is importance.

The conventional method of wound dressing process used gauze as the protection for external bacterial infection. However this method can cause the loss of protection when the outer surface of the dressing becomes moistened by wound exudate or external fluids (Jonathan and Zilberman, 2009). Compared to gauze dressing, the bacterial cellulose (BC) based bio composite has higher water holding capacity and water vapor transmission rate thus it can maintain the moist environment at the wound/dressing surface more longer than the conventional method. Another disadvantage of conventional dressing method is the discharge rate of the drug is very rapid causing the drug retaining capability is very low. With this new technique by using bacterial cellulose (BC) as the matrix, the drug containing capability can be increased due to the strong hydrogen bond between the carbonyl group from cellulose and hydroxyl group from the phenolic content in areca nut extract. This study will focus on the production of antibacterial bio composite from bacterial cellulose (BC) with areca nut extract. Areca nut is selected for the antibacterial materials based on the phenolic compound content in the nut. These combinations can be used in the application of wound healing.

1.3 RESEARCH OBJECTIVE

The main objective in this study is to produce antibacterial bio composite from bacterial cellulose (BC) and areca nut extract.

1.4 SCOPE

1. To fabricate the antibacterial bio composite from the bacterial cellulose (BC)(0wt. %, 7wt. %, 14wt. %, 21wt. % and 28wt. %) and areca nut extract (0%, 25%, 50%, 75% and 100%) by using casting method.

2. To characterize the characteristic of bio composite produced by using Water Absorption test, Phenolic content test, quantitative of tannin test, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), biodegradable by using *Aspergillus niger*, soil degradable test and testing for antibacterial activity by using Disc Diffusion assay.

1.5 RATIONAL AND SIGNIFICANCE

Being in line with the advanced lifestyles, the trends of pharmaceutical market in the world today also aware that there is a growing demand for instant remedies that easy to be used. People are exposed to get wound for each single time and they need an easy method to heal with a simple technique. To seize this opportunity, a compound or substance that has an ability to kill or slow down the growth of bacteria at specific wound is tended to be produced. Areca nut, is a seed of Areca palm (*Areca catechu*), has a potential to become antibacterial material because of the phenolic content contains in it (Buhler and Miranda, 2000). With a new invention of combination of starch and bacterial cellulose (BC) as biodegradable polymer with the antibacterial compound, it would give such an impressive market value product. Wound healing bio composite is not just good for pharmaceutical purposes, but also for the environment. This is due to its ability which can degrade by time.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Wound healing process needs to be take care from infection which can slow down the healing process. Thus, the production of antibacterial bio composite from bacterial cellulose (BC) and areca nut extract will be used as an alternative wound dressing to prevent this situation. Areca nut has been used in many places as the medicine because of its phenolic antioxidant attribute. A review is performed to identify studies that relevant to this study on production of antibacterial bio composite from bacterial cellulose (BC) and areca nut extract. This research is basically about the findings of an effective composition of bacterial cellulose (BC) and areca nut extract to produce antibacterial bio composite for wound healing process. Therefore this chapter provides reviews about the main component of this study and characterization of bio composite.

2.2 BACTERIAL CELLULOSE (BC)

Cellulose is the most abundant biopolymer on earth, recognized as the major component of plant biomass, but also a representative of microbial extracellular polymers. An efficient producer of cellulose is acetic acid bacteria *Acetobacter xylinum*. Several different techniques for bacterial cellulose (BC) production have been reported such as stationary culture, agitated culture, cultivation in the horizontal fermenters or cultivation in the internal-loop airlift reactors. Stationary cultivation methods produce pellicles of bacterial cellulose (BC) that being formed on the surface of the static culture (Son et al., 2003). Table 2.1 below shows the comparison between the results of various cultural conditions used by different researchers.

Table 2.1: The comparison between the results of various cultural conditions used by different researchers.

Volume (L)	Yield (g/L)	System	Temp. (°C)	pH	Time (h)	References
-	9.7	Shaking	-	-	7	Tsuchida et al. (1997)
30	20	Shaking	30	5	42	Kouda et al. (1998)
2	15	Shaking	30	5/5	50	Hwang et al. (1999)
Tubes	3	Static	30	5/6-7/5	4 weeks	Ishihara et al. (2002)
0.075	16.4	Shaking	30	5/6	192	Son et al. (2002)
0.61	21	Shaking	30	5	50	Naritomi et al. (2002)
0.1	12.8	Shaking	30	5	72	Bae et al. (2004)
0.03	-	Static	28	6	168	Keshk et al. (2005)

Source: Pourramezan et al. (2009)

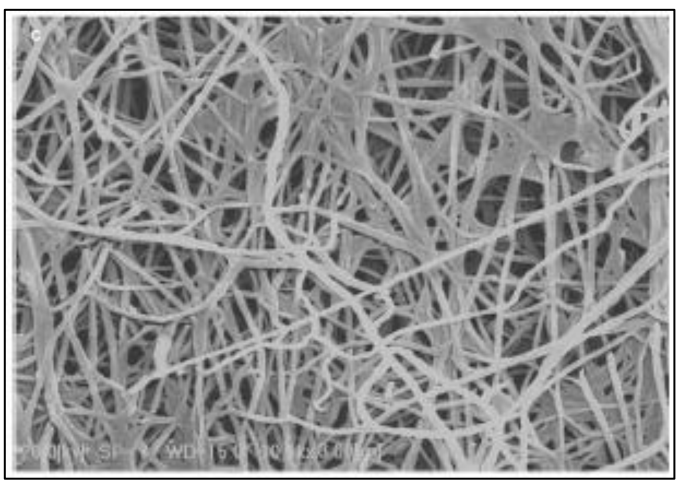

Figure 2.1: Scanning electron microscopy images of BC membrane from static culture of *Acetobacter xylinum.*

Source: Kim et al. (2010)

The bio composite produced from bacterial cellulose (BC) and starch present important advantages such as high availability, biodegradability, specific strength and modulus, sound attenuation, comparatively processing ability and low cost, energy consumption, density due to their flexibility and non-abrasive nature (Martins et al, 2009). The other researchers (Wan et al., 2007) also state that the bacterial cellulose produced by *Acetobacter xylinum* has unique, properties including high water-holding capacity, crystallinity, hydrophilicity, higher tensile strength and a pure ultra-fine fibre network structure. Based on research from Yano et al. (2005), the bacterial cellulose (BC) micro fibrils have a density of 1600 kg m−3, Young's modulus of 138 GPa, and tensile strength of at least 2 GPa.

Extensive research on bacterial cellulose (BC) revealed that it is chemically identical to plant cellulose (PC), but its macromolecular structure and properties are different. The molecular formula of bacterial cellulose (BC) $(C_6H_{10}O_5)_n$ is the same as the plant cellulose, but their physical and chemical features are different. Bacterial cellulose (BC) is preferred over the plant cellulose as it can be obtained in higher purity and exhibits a higher degree of polymerization, crystallinity index, tensile strength and water holding capacity compared with plant cellulose, making it more suitable as raw material for producing high fidelity acoustic speakers, high quality paper and dessert foods. Ongoing research of bacterial cellulose (BC) products includes a wide range of biomedical applications such as in treatment of chronic wounds and burns as temporary coverage, artificial cardiovascular tissues, and guided regeneration of bone, cartilage and nerve. Figure 2.2 shows the structure of bacterial cellulose (BC).

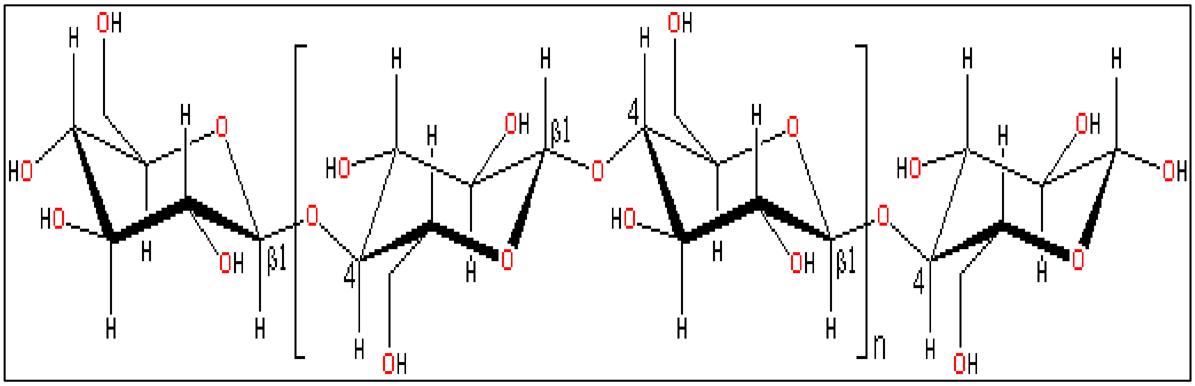

Figure 2.2: Bacterial cellulose structure

Source: http://www.lsbu.ac.uk/water/hycel.html (retrieve at 17 September 2011)

2.3 *ACETOBACTER XYLINUM*

Acetobacter xylinum is a gram negative bacterium and have used to synthesis of cellulose. According to Chawla et al. (2009), *Acetobacter xylinum* converts various carbon compounds, such as hexoses, glycerol, dihydroxyacetone, pyruvate, and dicarboxylic acids, into cellulose. The conversion is usually about 50% efficiency. Other gram-negative species like *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Sarcina*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella and Alcaligenes*can also produce bacterial cellulose. Based on research conducted by Ross et al. (1991), *Acetobacter xylinum* is most effective producers of cellulose. *Acetobacter xylinum* was cultured in coconut-water supplemented with 5.0% sucrose, 0.5% ammonium sulfate and 1.0% acetic acid. The culture media was incubated statically at 30°C for 7 days (Saibuatong and Phisalaphong, 2010). Based on research done by Krystynowic et al. (2005), the optimum temperature for *Acetobacter xylinum* growth is 25–30°C, and pH ranges from 5.4 to 6.2.

According to Bielecki et al. (2005), *Acetobacter xylinum* has been applied as a model microorganism for basic and applied studies on cellulose. It is an ability to produce relatively high levels of polymer from a wide range of carbon and nitrogen sources. It is a rod-shaped, aerobic, gram-negative bacterium that produces cellulose in the form of interwoven extracellular ribbons as part of primary metabolite. This bacterium grows and produces cellulose from a wide variety of substrates and is devoid of cellulose activity.

Klemma et al. (2001) has mentioned about the production of bacterial cellulose (BC) from *Acetobacter xylinum* in their research. The cellulose was constituted between the outer and the cytoplasma membrane. The cellulose was formed by synthesizing complexes or terminal complexes (TC) that are linearly arranged, and in association with pores at the surface of the bacterium. The glucan chain from the terminal complexes will elongates, where 6-8 glucan chain will be combined together. This combined glucan chain, as known as fibril, will elongates further as micro fibril, resembles as ribbon like structure. Multiple micro fibril will eventually be interwoven together, foaming matrix like constituents of pellicle, which is the bacterial cellulose (BC) membrane. Figure 2.3 illustrated the formation of bacterial cellulose (BC).

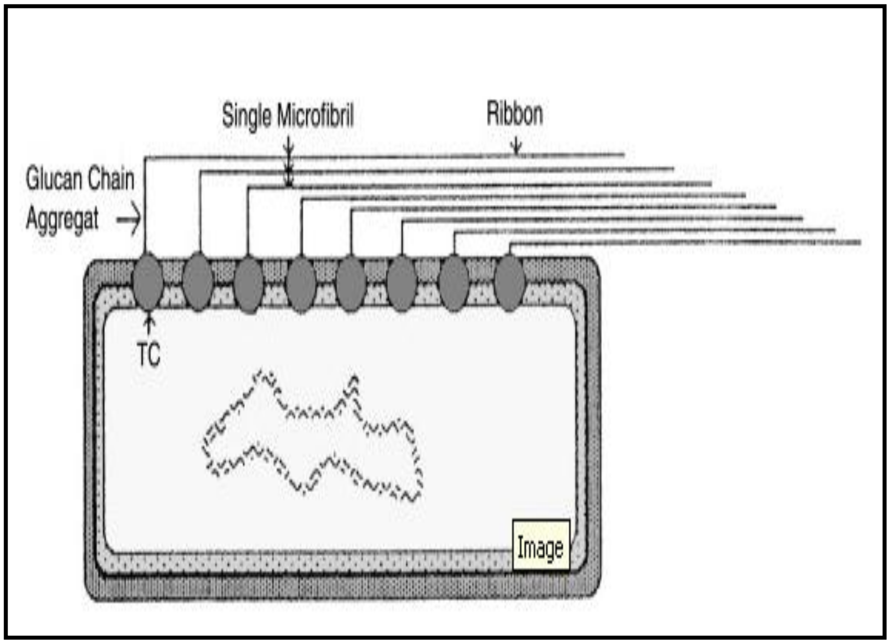

Figure 2.3: Formation of bacterial cellulose.

Source: Klemma et al. (2001)